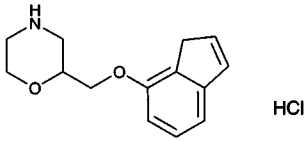
REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Indeloxazine hydrochloride

Molecular formula: $C_{14}H_{18}ClNO_2$
Molecular weight: 267.76
CAS Registry No.: 65043-22-3
Merck Index: 4972
Lednicer No.: 4 59



SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 300 ng/mL viloxazine in water + 500 μL 200 mM ammonium hydroxide + 4 mL ether, extract, centrifuge. Remove the organic layer and evaporate it to dryness at 45°, reconstitute the residue in 100 μL 3 mg/mL sodium bicarbonate and 200 μL 125 μg/mL dansyl chloride in acetone, heat at 45° for 20 min, cool, add 4 mL ether. Wash the ether solution twice with 3 mL water and evaporate it to dryness at 45°. Take up the residue in 100 μL n-heptane, inject a 3-5 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm LiChrosorb SI-60
Mobile phase: n-Heptane:ethyl acetate 20:3
Flow rate: 1.5
Injection volume: 3-5
Detector: F ex 365 em 505

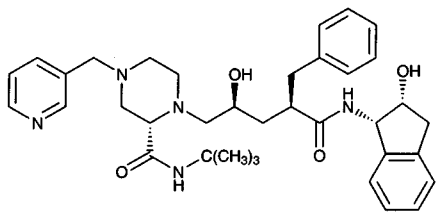
CHROMATOGRAM
Retention time: 3
Internal standard: viloxazine (4)
Limit of detection: 5 ng/mL

KEY WORDS
plasma; normal phase; derivatization

REFERENCE
Kamimura,H.; Sasaki,H.; Yokoi,K.; Kawamura,S. Determination of indeloxazine in plasma by liquid chromatography and gas chromatography-mass spectrometry, *J.Pharm.Sci.*, **1985**, *74*, 559–561.

Indinavir

Molecular formula: $C_{36}H_{47}N_5O_4$
Molecular weight: 613.80
CAS Registry No.: 150378-17-9, 157810-81-6 (sulfate)
Merck Index: 4979



SAMPLE
Matrix: blood
Sample preparation: 300 μL Plasma + 300 μL 50 mM pH 9.0 ammonium dihydrogen phosphate buffer + 30 μL 10.5 μg/mL IS in water, vortex for 10 s, add 3 mL diethyl ether, vortex for 30

s, keep at -20° for 30 min. Remove the ether layer and evaporate it to dryness under nitrogen. Reconstitute the residue with 150 μL 10 mM pH 5.5 ammonium dihydrogen phosphate buffer, centrifuge at 750 g for 5 min, inject a 35 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Delta-pak C4 (Waters)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mM ammonium dihydrogen phosphate and 1 mM 1-heptanesulfonic acid sodium salt, pH adjusted to 4.8 with ammonium hydroxide.)
Flow rate: 0.6
Injection volume: 35
Detector: UV 210

CHROMATOGRAM
Retention time: 13.2 ± 0.5
Internal standard: methylindinavir (methyl at 5 position on pyridine) (14.1 ± 0.7)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: nelfinavir, ritonavir, saquinavir
Noninterfering: didanosine, lamivudine, stavudine, zalcitabine, zidovudine

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Iayewardene,A.L.; Zhu,F.; Aweeka,F.T.; Gambertoglio,J.G. Simple high-performance liquid chromatographic determination of the protease inhibitor indinavir in human plasma, *J.Chromatogr.B*, **1998**, *707*, 203–211.

SAMPLE
Matrix: blood
Sample preparation: Add 50 μL 1 μg/mL IS in MeCN:water 50:50 to 1 mL plasma and vortex. Add 1 mL 100 mM pH 9.5 borate buffer, vortex, add 8 mL isopropanol:chloroform 1:15 (Caution! Chloroform is a carcinogen!), mix on a flat-bed shaker for 15 min, centrifuge at 1500 g for 5 min. Remove the lower organic layer and evaporate it under a stream of nitrogen at 45°. Reconstitute the residue in 1 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 50 × 3.9 5 μm Symmetry C8
Mobile phase: MeCN:water 40:60 containing 2 mM ammonium acetate
Flow rate: 1
Injection volume: 50
Detector: MS, PE-SCIEX API IIIplus triple quadrupole, heated nebulizer, corona discharge needle (+4.5 μA), nebulizer 500°, collision gas argon at 260 × 10^{12} atoms/cm^2, nebulizing gas nitrogen at 80 psi and 0.6 mL/min, orifice +75 V, electron multiplier -4.0 kV, dwell time 400 ms with a 30 ms pause time between scans, Q1 at m/z 523, Q2 at m/z 512, Q3 at m/z 273

CHROMATOGRAM
Retention time: 1.5
Internal standard: structure given in paper
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Woolf,E.; Haddix,H.M.; Matuszewski,B. Determination of an in vivo metabolite of a human immunodeficiency virus protease inhibitor in human plasma by high-performance liquid chromatography with tandem mass spectrometry, *J.Chromatogr.A*, **1997**, *762*, 311–319.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL MeCN:water 50:50 + 25 μL 1 μg/mL IS in MeCN, vortex, add 1 mL 100 mM pH 9.5 borate buffer, vortex, add 8 mL MTBE, mix on a Glas Col (Terre Haute, IN) RD 350 rotator for 15 min, centrifuge at 1500 g for 5 min, freeze the aqueous phase in a dry ice-acetone bath. Decant the organic layer and evaporate it under a stream of nitrogen at 42°. Reconstitute the residue in 175 μL mobile phase, inject a 6 μL aliquot.

HPLC VARIABLES
Column: 50 × 2 3 μm BDS Hypersil C8
Mobile phase: MeCN:water 40:60 containing 7 mM pH ammonium acetate, adjusted to pH 4.9 with formic acid
Flow rate: 0.2
Injection volume: 6
Detector: MS, PE-Sciex API IIIplus triple quadrupole, turbo-ion spray, turbo probe at 500°, auxiliary gas nitrogen, nebulizing gas nitrogen at 80 psi, positive ion mode, interface sprayer at +4 kV, sampling orifice at +60 V, m/z 614

CHROMATOGRAM
Retention time: 2.6
Internal standard: indinavir analog (4.2)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: hexadeuterated indinavir (m/z 620)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Woolf,E.J.; Matuszewski,B.K. Simultaneous determination of unlabeled and deuterium-labeled indinavir in human plasma by high-performance liquid chromatography ith tandem mass spectrometric detection, *J.Pharm.Sci.*, **1997**, *86*, 193–198.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add four volumes of MeCN to microsomal incubation, centrifuge at 1500 g for 10 min, evaporate supernatant to dryness under nitrogen at 40°, reconstitute the residue in 100-120 μL mobile phase, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Hypersil 5C18 (Phenomenex)
Mobile phase: Gradient. A was MeCN. B was 0.3% pH 6 triethylamine in water. A:B from 25:75 to 45:55 over 18 min
Flow rate: 1.5
Injection volume: 80
Detector: UV 240

CHROMATOGRAM
Retention time: 16.1

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

REFERENCE
Koudriakova,T.; Iatsimirskaia,E.; Utkin,I.; Gangl,E.; Vouros,P.; Storozhuk,E.; Orza,D.; Marinina,J.; Gerber,N. Metabolism of the human immunodeficiency virus protease inhibitors indinavir and ritonavir by human intestinal microsomes and expressed cytochrome P4503A4/3A5: Mechanism-based inactivation of cytochrome P4503A by ritonavir, *Drug Metab.Dispos.*, **1998**, *26*, 552–561.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add four volumes of 1-chlorobutane containing 50 ng/mL 5-methoxypsoralen to microsomal incubation, mix vigorously, centrifuge at 1500 g for 10 min, evaporate 1-chlorobutane extract to dryness under nitrogen, reconstitute the residue in 100 μL mobile phase, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Hypersil 5C18 (Phenomenex)
Mobile phase: Gradient. A was MeCN. B was 0.3% pH 6 triethylamine in water. A:B from 25:75 to 45:55 in 18 min
Flow rate: 1.5
Injection volume: 80
Detector: UV 240

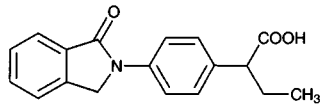
CHROMATOGRAM
Retention time: 16.1
Internal standard: 5-methohypsoralen (14.6)
Limit of detection: 30 nM

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

REFERENCE
Koudriakova,T.; Iatsimirskaia,E.; Utkin,I.; Gangl,E.; Vouros,P.; Storozhuk,E.; Orza,D.; Marinina,J.; Gerber,N. Metabolism of the human immunodeficiency virus protease inhibitors indinavir and ritonavir by human intestinal microsomes and expressed cytochrome P4503A4/3A5: Mechanism-based inactivation of cytochrome P4503A by ritonavir, *Drug Metab.Dispos.*, **1998**, *26*, 552–561.

Indobufen



Molecular formula: $C_{16}H_{17}NO_3$
Molecular weight: 295.34
CAS Registry No.: 63610-08-2
Merck Index: 4991

SAMPLE
Matrix: blood
Sample preparation: 0.5-1 mL Plasma + 500 μL water + 100 μL 600 mM sulfuric acid + 40 mg NaCl + 4 mL diethyl ether, extract on a rotamixer, centrifuge at 1200 g. Remove the organic layer and evaporate it to dryness under a stream of air at 30°, add 5 drops toluene, evaporate to dryness under a stream of air at 30°, reconstitute the residue in 200 μL 50 mM triethylamine in MeCN, add 100 μL 60 mM ethyl chloroformate in MeCN, after 30 s add 100 μL 1 M l-leucinamide hydrochloride in MeOH containing 1 M triethylamine, after 2 min add 500 μL 250 mM HCl, extract with 4 mL ethyl acetate. Evaporate the organic layer to dryness under a stream of air at 30°, reconstitute the residue with 500 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4 Perisorb RP-18 (Merck)
Column: 250 × 4 7 μm LiChroCart RP-18 (Merck)
Mobile phase: MeCN:10 mM pH 6.5 phosphate buffer 38:62
Flow rate: 2
Injection volume: 10
Detector: UV 275

CHROMATOGRAM
Retention time: 6 (-), 8 (+) (assignment tentative)
Internal standard: indobufen

OTHER SUBSTANCES
Extracted: indoprofen

KEY WORDS
plasma; derivatization; chiral; indobufen is IS

REFERENCE
Björkman,S. Determination of the enantiomrs of indoprofen in blood plasma by high-performance liquid chromatography after rapid derivatization by means of ethyl chloroformate, *J.Chromatogr.*, **1985**, *339*, 339–346.

SAMPLE
Matrix: blood
Sample preparation: 0.5-1 mL Plasma + 100 μL 600 mM sulfuric acid + 40 mg NaCl + 4 mL diethyl ether, extract by rotation, centrifuge at 1200 g. Remove the organic layer and evaporate it to dryness under a stream of air at 30°, add 5 drops of toluene, evaporate to dryness under a stream of air. Reconstitute the residue in 200 μL 50 mM triethylamine in MeCN, add 100 μL 60 mM ethyl chloroformate in MeCN, let stand for 30 s, add 100 μL 1 M leucinamide hydrochloride in MeOH containing 1 M triethylamine, let stand for 2 min, add 500 μL 250 mM HCl, extract with 4 mL ethyl acetate. Remove the organic layer and evaporate it to dryness under a stream of air at 30°, reconstitute in 500 μL MeOH, inject a 10 μL aliquot (J.Chromatogr. 1985, 339, 339).

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:pH 6.4 phosphate buffer 40:60
Flow rate: 1.5
Injection volume: 10
Detector: UV 275

CHROMATOGRAM
Retention time: 6.977 (R), 8.848 (S)

KEY WORDS
chiral; derivatization

REFERENCE
Perrone,G.; Farina,M. High-performance liquid chromatographic method for direct resolution of the indobufen enantiomeric components, *J.Chromatogr.*, **1990**, *520*, 373–378.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: Hexane:isopropanol:formic acid 80:20:0.5
Flow rate: 1.5
Detector: UV 270

CHROMATOGRAM
Retention time: 8.20 (R), 10.91 (S)
Limit of detection: 2 ng

KEY WORDS
chiral; $\alpha = 1.33$

REFERENCE
Perrone,G.; Farina,M. High-performance liquid chromatographic method for direct resolution of the indobufen enantiomeric components, *J.Chromatogr.*, **1990**, *520*, 373–378.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + 50 μL 5000 U/mL β-glucuronidase (Sigma) + 2 mL 100 mM pH 5 acetate buffer, heat at 37° for 16 h, add S-indoprofen, add 1 mL 1 M HCl, extract with diethyl ether. Remove the organic phase and extract it with 500 μL 1 M NaOH. Remove the aqueous phase and add it to 1 mL 1 M HCl, extract with diethyl ether. Remove the organic layer and evaporate it to dryness under a stream of air at 30°, add 5 drops of toluene, evaporate to dryness under a stream of air. Reconstitute the residue in 200 μL 50 mM triethylamine in MeCN, add 100 μL 60 mM ethyl chloroformate in MeCN, let stand for 30 s, add 100 μL 1 M leucinamide hydrochloride in MeOH containing 1 M triethylamine, let stand for 2 min, add 500 μL 250 mM HCl, extract with 4 mL ethyl acetate. Remove the organic layer and evaporate it to dryness under a stream of air at 30°, reconstitute in 500 μL MeOH, inject a 10 μL aliquot (J.Chromatogr. 1985, 339, 339).

HPLC VARIABLES
Column: 125 × 4 4 μm Lichrocart Superspher (Merck)
Mobile phase: MeCN:10 mM pH 6.5 phosphate buffer 35:65
Injection volume: 10
Detector: UV 275

CHROMATOGRAM
Internal standard: indoprofen
Limit of detection: 5 μg/mL

KEY WORDS
chiral; derivatization; pharmacokinetics

REFERENCE
Strolin Benedetti,M.; Frigerio,E.; Tamassia,V.; Noseda,G.; Caldwell,J. The dispositional enantioselectivity of indobufen in man, *Biochem.Pharmacol.*, **1992**, *43*, 2032–2034.

SAMPLE
Matrix: urine
Sample preparation: Inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 LiChrosorb C18
Mobile phase: Gradient. MeCN:water 25:75 containing 0.1% trifluoroacetic acid for 30 min then MeCN:water 40:60 containing 0.1% trifluoroacetic acid for 20 min (step gradient).
Flow rate: 2
Detector: F ex 290 em 440

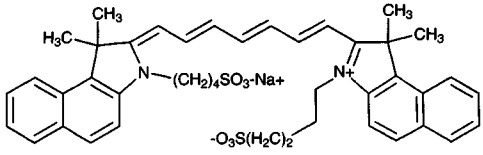
CHROMATOGRAM
Retention time: 36.8

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; mouse

REFERENCE
Grubb,N.; Caldwell,J.; Strolin-Benedetti,M. Excretion balance and urinary metabolism of indobufen in rats and mice, *Biochem.Pharmacol.*, **1993**, *46*, 759–761.

Indocyanine green



Molecular formula: $C_{43}H_{47}N_2NaO_6S_2$
Molecular weight: 774.98
CAS Registry No.: 3599-32-4
Merck Index: 4992

SAMPLE

Matrix: blood
Sample preparation: 0.5 mL Plasma +10 μL 250 μg/mL 1-acetamidopyrene in MeOH + 200 μL 1 M ammonium sulfate + 800 μL cold MeCN, vortex for 30 s, store at -20° for at least 30 min, vortex, centrifuge at 1500 g for 30 min. Remove 400 μL of the upper organic layer and evaporate it under a stream of nitrogen. Reconstitute with 100 μL mobile phase, vortex for 30 s, inject a 75 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 47:53 (Buffer was 6.805 g potassium monophosphate in 1 L water, adjust pH to 6.00 with 10 M NaOH.)
Flow rate: 1
Injection volume: 75
Detector: UV 214

CHROMATOGRAM

Retention time: 7.2
Internal standard: 1-acetamidopyrene (9.7)
Limit of detection: 39 ng/mL

OTHER SUBSTANCES

Simultaneous: lorazepam, antipyrine
Noninterfering: adenosine, albuterol, alphenal, aspirin, caffeine, carbamazepine, cefazolin, cephalexin, cephalothin, cimetidine, ciprofloxacin, claforan, desipramine, enoxacin, fleroxacin, furosemide, hydralazine, hydrochlorothiazide, minoxidil, norfloxacin, phenytoin, propafenone, sulindac, teicoplanin, theophylline, vancomycin

KEY WORDS

plasma

REFERENCE

Awni,W.M.; Bakker,L.J. Antipyrine, indocyanine green, and lorazepam determined in plasma by high-pressure liquid chromatography, *Clin.Chem.*, **1989**, *35*, 2124–2126.

SAMPLE

Matrix: blood
Sample preparation: 25 μL Plasma + 25 μL cold (-20°) 60 μg/mL IS in acetone, vortex for 30 s, centrifuge at 13000 g for 1 min, inject a 10-20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 5 μm octylsilane (Alltech)
Mobile phase: MeCN:50 mM pH 4.0 phosphate buffer 42:58
Flow rate: 1.5
Injection volume: 10-20
Detector: F ex 214 em 370

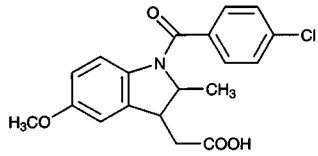
CHROMATOGRAM

Retention time: 7.3
Internal standard: 1,1',3,3,3',3'-hexamethyl-4,4',5,5'-dibenzo-2,2'-indotricarbocyanine perchlorate (Eastman Kodak) (8.7)
Limit of detection: 500 ng/mL

KEY WORDS
rat; plasma; pharmacokinetics

REFERENCE
Pollack,G.M.; Brouwer,K.L.R.; Demby,K.B.; Jones,J.A. Determination of hepatic blood flow in the rat using sequential infusions of indocyanine green or galactose, *Drug Metab.Dispos.*, **1990**, *18*, 197–202.

Indomethacin



Molecular formula: $C_{19}H_{16}ClNO_4$
Molecular weight: 357.79
CAS Registry No.: 53-86-1, 74252-25-8 (sodium salt trihydrate)
Merck Index: 4998
Lednicer No.: 1 318; 2 345; 3 165

SAMPLE
Matrix: aqueous humor
Sample preparation: 100 μL Aqueous humor + 500 μL MeCN + 30 μL 400 ng/mL (+)-naproxen in MeOH, mix mechanically for 90 s, centrifuge at 3000 g for 20 min. Remove the supernatant and dry it under nitrogen at room temperature, dissolve the residue in 50 μL mobile phase by swirl-mixing for 1 min, centrifuge at 3000 g for 20 s, reduce volume to 20-30 μL, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.5 5 μm Ultrasphere octyl
Mobile phase: MeCN:triethylamine:1.65% glacial acetic acid 505:0.65:495, pH 4.35
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 6.64
Internal standard: naproxen (3.89)

OTHER SUBSTANCES
Extracted: diclofenac, flurbiprofen, meclofenamic acid
Simultaneous: bacitracin, cortisone acetate, diazepam, fluorometholone, hydrocortisone acetate, imipramine, ketoprofen, ketorolac tromethamine, levobunolol, metipranolol, neomycin, prednisolone acetate, proparacaine, propranolol, salicylic acid, sulfacetamide, suprofen
Noninterfering: acebutolol, acetaminophen, acetazolamide, alprenolol, apraclonidine, atenolol, atropine, betamethasone, betaxolol, bupivacaine, caffeine, cyclopentolate, dexamethasone, diphenhydramine, erythromycin, haloperidol, lidocaine, phenylephrine, polymyxin B, procaine, scopolamine, timolol, tropicamide

KEY WORDS
human; rabbit

REFERENCE
Riegel,M.; Ellis,P.P. High-performance liquid chromatography assay for antiinflammatory agents diclofenac and flurbiprofen in ocular fluids, *J.Chromatogr.B*, **1994**, *654*, 140–145.

SAMPLE
Matrix: aqueous humor
Sample preparation: 200 μL Aqueous humor + 200 μL MeCN, vortex for 15 s, centrifuge at 2000 rpm for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 5 μm Lichrospher 100 C18
Column: 125 × 4 5 μm Lichrospher 100 C18
Mobile phase: MeOH:50 mM pH 3 sodium phosphate buffer 65:35
Flow rate: 1
Injection volume: 20
Detector: E, Shimadzu L-ECD-6A, glassy carbon working electrode 1.20 V, Ag/AgCl reference electrode or UV 266

CHROMATOGRAM
Retention time: 7.5
Limit of detection: 50 ng/mL (UV), 20 ng/mL (E)

KEY WORDS
rabbit

REFERENCE
Baudrit,O.; Fabre,H. Evaluation of electrochemical and fluorescence detection in liquid chromatography for the assay of indomethacin in aqueous humor samples, *J.Liq.Chromatogr.*, **1995**, *18*, 3283–3299.

SAMPLE
Matrix: bile, blood, gastric contents, urine
Sample preparation: Plasma. 300 μL Plasma + 1 mL MeCN, vortex for 30 s, centrifuge at 3500 rpm. Remove the supernatant and evaporate it to 100 μL under a stream of nitrogen at 50°, inject a 20 μL aliquot. Urine, bile, gastric fluid. 300 μL Urine, bile, or gastric fluid + 100 μL 5 M NaOH, let stand at room temperature for 15 min, adjust the pH with 100 μL 28.3% phosphoric acid, add 1 (urine, bile) or 1.5 (gastric fluid) mL MeCN, vortex for 30 s, centrifuge at 3500 rpm. Remove the supernatant and evaporate it to 100 μL under a stream of nitrogen at 50°, inject a 20 (urine), 35 (bile), or 40 (gastric fluid) μL aliquot. (Borate buffer was 12.4 g boric acid and 10 mL 1 M NaOH made up to 1 L with water, pH 7.2.)

HPLC VARIABLES
Guard column: Microguard reverse-phase (Bio-Rad)
Column: 100 × 8 Radial-PAK C18 in a radial compression module
Mobile phase: MeCN:buffer 70:30 (Buffer was 6.8 g/L KH_2PO_4 adjusted to pH 3.0 with 85% phosphoric acid.)
Flow rate: 2
Injection volume: 20-40
Detector: UV 340

CHROMATOGRAM
Retention time: 13
Internal standard: indomethacin

OTHER SUBSTANCES
Extracted: sulindac

KEY WORDS
plasma; indomethacin is IS

REFERENCE
Musson,D.G.; Vincek,W.C.; Constanzer,M.L.; Detty,T.E. Analytical methods for the determination of sulindac and metabolites in plasma, urine, bile, and gastric fluid by liquid chromatography using ultraviolet detection, *J.Pharm.Sci.*, **1984**, *73*, 1270–1273.

SAMPLE
Matrix: blood
Sample preparation: Mix 500 μL serum with 100 μL MeOH:50 mM pH 3 sodium phosphate buffer 50:50, vortex for 5 s. Add 1 mL MeCN, vortex for 1 min. Centrifuge the mixture at 14000 rpm for 5 min, decant the clear upper layer. Add 500 μL MeCN to the pellet, mix, centrifuge at 14000 rpm for 5 min. Combine the upper layers and evaporate at 40°. Reconstitute the

residue with 300 μL MeOH:50 mM pH 3.0 sodium phosphate buffer 40:60 containing 0.5% sodium metabisulfate (sic). Vortex for 30 s and inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Techsil C18 (HPLC Technology, Macclesfield)
Mobile phase: MeCN:50 mM phosphate buffer 46:63, adjusted to pH 3.0 with NaOH
Flow rate: 1.2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 7.17
Internal standard: indomethacin

OTHER SUBSTANCES
Extracted: sulindac

KEY WORDS
indomethacin is IS; serum

REFERENCE
Kanfer,I.; Brown,C.; Koninigs,M.; Swarbrick,J. Absorption of sulindac from a novel (Pro-SorbTM) liquid formulation, *Biopharm.Drug Dispos.*, **1996**, *17*, 209–221.

SAMPLE
Matrix: blood
Sample preparation: Precipitate 100 μL serum with 200 μL 2 μg/mL IS in MeCN, centrifuge at 12 000 g for 5 min. Inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChroCART LiChrospher 60 RP Select B
Column: 125 × 4 5 μm LiChroCART LiChrospher 60 RP Select B
Mobile phase: MeCN:buffer 60:40 (Buffer was 25 mM pH 3.0 triethylammonium phosphate containing 2% MeCN.)
Flow rate: 1
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 2.28
Internal standard: mefenamin (2.94)
Limit of detection: 110 ng/mL

KEY WORDS
serum

REFERENCE
Hannak,D.; Scharbert,F.; Kattermann,R. Stepwise binary gradient high-performance liquid chromatographic system for routine drug monitoring, *J.Chromatogr.A*, **1996**, *728*, 307–310.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 250 μL 1 M sulfuric acid + 5 mL 24 ng/mL p-phenylphenol in dichloromethane, vortex for 10 s, centrifuge at 500 g for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject a 20-30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax ODS
Mobile phase: Gradient. MeOH:100 mM pH 5 acetate buffer 45:55 for 3 min, to 62:38 over 2 min, maintain at 62:38 for 10 min.

Column temperature: 40
Flow rate: 1.5
Injection volume: 20-30
Detector: UV 254

CHROMATOGRAM
Retention time: 13.83
Internal standard: p-phenylphenol (12.43)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: acetaminophen, caffeine, carbamazepine, ethosuximide, fenoprofen, naproxen, phenobarbital, phenytoin, primidone, quinidine, salicylic acid, sulindac, theophylline, tolmetin, valproic acid
Noninterfering: ibuprofen

KEY WORDS
plasma

REFERENCE
Shimek,J.L.; Rao,N.G.S.; Wahba Khalil,S.K. High performance liquid chromatographic analysis of tolmetin, indomethacin and sulindac in plasma, *J.Liq.Chromatogr.*, **1981**, *4*, 1987–2013.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 100 μL pH 2 dilute sulfuric acid + 1 mL MeCN, vortex for 30 s, centrifuge at 2500 rpm for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax CN
Mobile phase: MeCN:4% aqueous acetic acid 45:55
Flow rate: 1
Injection volume: 10
Detector: UV 340

CHROMATOGRAM
Retention time: 6
Internal standard: indomethacin

OTHER SUBSTANCES
Extracted: sulindac

KEY WORDS
plasma; indomethacin is IS

REFERENCE
Clark,C.R.; McMillian,C.L.; Hoke,J.F.; Campagna,K.D.; Ravis,W.R. Liquid chromatographic determination of sulindac and metabolites in serum, *J.Chromatogr.Sci.*, **1987**, *25*, 247–251.

SAMPLE
Matrix: blood
Sample preparation: Activate a 1 mL Bond-Elut C8 SPE cartridge with 2 mL MeOH then 1 mL 10 mM HCl, do not allow it to dry completely. Sonicate 1 mL whole blood for 20-30 min then apply to cartridge. Wash with 100 μL water, elute with three 500 μL portions of MeOH:MeCN:1% aqueous ammonium hydroxide 50:20:30, combine eluents and evaporate to dryness under a stream of nitrogen at 40°. Redissolve in 1 mL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Spherisorb ODS
Mobile phase: MeCN:MeOH:buffer 35:13:52 (Buffer was water adjusted to pH 3.2 with orthophosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 250

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: ketoprofen, acetaminophen, salicylic acid, naproxen, fenoprofen, ibuprofen

KEY WORDS
whole blood; SPE

REFERENCE
Moore,C.M.; Tebbett,I.R. Rapid extraction of anti-inflammatory drugs in whole blood for HPLC analysis, *Forensic Sci.Int.*, **1987**, *34*, 155–158.

SAMPLE
Matrix: blood
Sample preparation: Wash a Sep-Pak C18 cartridge with 2 mL MeOH, 5 mL water, and 1 mL 0.25 mM pH 3.0 ammonium phosphate buffer. 20-200 μL Plasma + 100 μL MeOH + 20 μL 50 μg/mL indomethacin in MeOH + 100 μL 0.25 mM pH 3.0 ammonium phosphate buffer + 100 μL water, vortex for 2 min, centrifuge at 1800 g for 10 min. Add the supernatant to the cartridge, wash with 5 mL water, elute twice with 5 mL portions of MeOH. Evaporate eluate to dryness under vacuum, dissolve the residue in 1 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Brownlee RP18
Mobile phase: MeOH:buffer 75:25 (Buffer prepared by diluting 0.25 mM ammonium phosphate buffer adjusted to pH 3.0 with orthophosphoric acid.)
Injection volume: 20
Detector: E ESA Coulochem Model 5100 A, + 0.9 V

CHROMATOGRAM
Retention time: 14.6
Internal standard: naproxen (10.0)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Also analyzed: sulindac, piroxicam, diflunisal

KEY WORDS
plasma

REFERENCE
Kazemifard,A.G.; Moore,D.E. Liquid chromatography with amperometric detection for the determination of non-steroidal anti-inflammatory drugs in plasma, *J.Chromatogr.*, **1990**, *533*, 125–132.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 1 M pH 10.0 Tris buffer containing 150 ng/mL indomethacin + 8 mL diethyl ether, vortex 3 min, freeze for 1 h. Remove organic phase and evaporate it to dryness at room temperature. Dissolve residue in 200 μL MeOH, inject 20 μL aliquot.

HPLC VARIABLES
Column: 70 × 4.6 3 μm Ultrasphere XL ODS

Mobile phase: MeOH:20 mM ammonium acetate buffer (pH 5.0) 65:35
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 1.64
Internal standard: indomethacin
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: dipyridamole

KEY WORDS
plasma; indomethacin is IS

REFERENCE
Barberi,M.; Merlin,J.L.; Weber,B. Sensitive determination of free and plasma protein-bound dipyridamole by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *565*, 511–515.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut phenyl SPE cartridge with 5 mL MeOH and 5 mL water. Adjust pH of 500 μL plasma to 3.4 with 345 mM citrate buffer, add to SPE cartridge, wash with water, dry, elute with 5 mL hexane:diethyl ether 50:50. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 6 × 4 4 μm Nova-Pack C18
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:20 mM ammonium sulfate 55:45
Flow rate: 1.5
Injection volume: 25
Detector: UV 340

CHROMATOGRAM
Retention time: 7.5
Internal standard: indomethacin

OTHER SUBSTANCES
Extracted: phenylbutazone, oxyphenbutazone, suxibuzone

KEY WORDS
plasma; SPE; indomethacin is IS

REFERENCE
Caturla,M.C.; Cusido,E. Solid-phase extraction for the high-performance liquid chromatographic determination of indomethacin, suxibuzone, phenylbutazone and oxyphenbutazone in plasma, avoiding degradation of compounds, *J.Chromatogr.*, **1992**, *581*, 101–107.

SAMPLE
Matrix: blood
Sample preparation: 20 μL serum + 20 μL MeCN, vortex for a few s, centrifuge at 10000 g for 2 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:25 mM phosphate buffer 35:65 containing 30 mM hydrogen peroxide, adjusted to pH 7.0 with 1 M NaOH

Flow rate: 1
Injection volume: 20
Detector: F ex 358 em 462 following post-column reaction. The column effluent flowed through a 15 m × 0.5 mm ID stainless steel coil at 180° then a 3 m × 0.5 mm ID stainless steel coil at 15° to the detector.

CHROMATOGRAM
Retention time: 10
Limit of detection: 500 ng/mL

KEY WORDS
post-column reaction; serum; pharmacokinetics

REFERENCE
Kubo,H.; Umiguchi,Y.; Kinoshita,T. Fluorometric determination of indomethacin in serum by high performance liquid chromatography using in-line oxidation with hydrogen peroxide, *J.Liq.Chromatogr.*, **1993**, *16*, 465–474.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma + 250 μL 0.75 μg/mL mefenamic acid in MeCN + 50 μL MeCN, vortex, centrifuge at 9000 g for 3 min. Remove 250 μL of the supernatant and evaporate it to dryness under vacuum, dissolve the residue in 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 Alltech 5 μm C18 bonded-phase silica
Column: 250 × 4.6 Vydac column packed with Merck 5 μm C18 bonded-phase silica
Mobile phase: MeCN:10 mM phosphoric acid 60:40, pH 2.6
Flow rate: 0.9
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 6.3
Internal standard: mefenamic acid (9.2)
Limit of detection: 60 ng/mL

KEY WORDS
plasma

REFERENCE
Niopas,I.; Mamzoridi,K. Determination of indomethacin and mefenamic acid in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *656*, 447–450.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 60 μm Separon SGX C18 SPE cartridge with 5 mL MeOH, 5 mL water, and 5 mL buffer. 250 μL Blood + 500 μL water, shake for 5 min, sonicate for 5 min, let stand at room temperature for 5 min, add 1 mL buffer, shake for 5 min, centrifuge at 1930 g for 10 min, add supernatant to cartridge, wash with 5 mL buffer, wash with 10 mL water, dry with vacuum for 5 min, elute with dichloromethane. Evaporate eluate to dryness under a stream of nitrogen, dissolve in 100 μL mobile phase, inject 10 μL aliquot. (Buffer was 66 mM KH_2PO_4 adjusted to pH 2.0 with phosphoric acid.)

HPLC VARIABLES
Column: 150 × 3.3 5 μm Separon SGX C18 glass column
Mobile phase: MeOH water 220:100, adjusted to pH 3.0 with 5% perchloric acid
Flow rate: 1.3
Injection volume: 10
Detector: UV 222

CHROMATOGRAM
Retention time: 7.8
Internal standard: indomethacin

OTHER SUBSTANCES
Simultaneous: ibuprofen

KEY WORDS
SPE; indomethacin is IS; rabbit; human

REFERENCE
Sochor,J.; Klimes,J.; Zahradnicek,M.; Sedlacek,J. High-performance liquid chromatographic assay for ibuprofen in whole blood using solid-phase extraction, *J.Chromatogr.B*, **1994**, *654*, 282–286.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond-Elut C2 SPE cartridge with 1 mL MeOH and 1 mL mobile phase. 1 mL Serum + 1 drop saturated ammonium sulfate solution + 1 drop 1 M HCl, vortex for 3 min, add to the SPE cartridge, wash with six 500 μL portions of wash solvent, elute with four 250 μL aliquots of mobile phase, combine the eluates, vortex, inject a 100 μL aliquot. (Wash solvent was MeCN:water adjusted to pH 3.0 with phosphoric acid 20:80.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere C8
Mobile phase: MeCN:68 mM pH 2.5 phosphate buffer 55:45
Flow rate: 0.5
Injection volume: 100
Detector: F ex 232 em 335 (filter) following post-column photolysis. The effluent from the column flowed through a 7.9 m × 0.3 mm i.d. coil of PTFE irradiated by an SC3-9 UV lamp (UVP) (cooled with air) to the detector.

CHROMATOGRAM
Retention time: 12
Internal standard: indomethacin

OTHER SUBSTANCES
Extracted: sulindac

KEY WORDS
serum; post-column reaction; SPE; indomethacin is IS; post-column photochemical derivatization

REFERENCE
Siluveru,M.; Stewart,J.T. Determination of sulindac and its metabolites in human serum by reversed-phase high-performance liquid chromatography using on-line post-column ultraviolet irradiation and fluorescence detection, *J.Chromatogr.B*, **1995**, *673*, 91–96.

SAMPLE
Matrix: blood
Sample preparation: Erythrocytes. 500 μL Erythrocytes + 900 μL water, shake for 5 min, sonicate for 5 min, let stand at room temperature for 5 min, add 400 μL 3 M HCl, shake for 5 min, add 6 mL dichloromethane, shake, centrifuge at 1930 g for 5 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 10 μL aliquot. Plasma. Acidify 500 μL plasma gradually with 900 μL 1 M HCl, shake, add 200 μL 3 M HCl, shake for 5 min, add 6 mL dichloromethane, shake, centrifuge at 1930 g for 5 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.3 5 μm C18 glass column (Tessek)
Mobile phase: MeOH:water 66:30 adjusted to pH 3.0 with 5% perchloric acid

Flow rate: 1.3
Injection volume: 10
Detector: UV 222

CHROMATOGRAM
Retention time: 7.8
Internal standard: indomethacin

OTHER SUBSTANCES
Extracted: ibuprofen
Simultaneous: diazepam, phenylanthranilic acid

KEY WORDS
plasma; erythrocytes; rabbit; indomethacin is IS

REFERENCE
Sochor,J.; Klimes,J.; Sedlácek,J.; Zahradnicek,M. Determination of ibuprofen in erythrocytes and plasma by high-performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 899–903.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 253

CHROMATOGRAM
Retention time: 8.91
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; bupren-

orphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF, gastric contents, urine

Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)

Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.

Column temperature: 50

Flow rate: 1.5

Detector: UV 220

CHROMATOGRAM

Retention time: 15.27

Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS

serum; column-switching

REFERENCE

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE

Matrix: blood, tissue

Sample preparation: Plasma. 50 μL Plasma + 150 μL 8 μg/mL mefenamic acid in MeOH, mix, centrifuge at 15000 rpm, filter (0.45 μm), inject an aliquot. Tissue. Homogenize liver in ice-cold 10 mM pH 7.4 phosphate buffer, 1 mL homogenate + 2 mL 8 μg/mL mefenamic acid in MeOH, mix, centrifuge at 15000 rpm, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:MeOH:water:acetic acid 65:10:25:1
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Internal standard: mefenamic acid
Limit of detection: 30 ng/mL

KEY WORDS
plasma; rat; liver

REFERENCE
Ogiso,T.; Iwaki,M.; Kinoshita,T.; Tanino,T.; Paku,T. Pharmacokinetics of indomethacin octyl ester (prodrug) and indomethacin produced from the prodrug, *J.Pharm.Sci.*, **1994**, *83*, 34–37.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 50 μL 500 ng/mL mefenamic acid + 1 mL 100 mM HCl + 10 mL dichloromethane, rotate for 10 min, centrifuge at 1500 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°. Redissolve the residue in mobile phase, inject a 20 μL aliquot. Urine. 50 μL Urine + 1 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 75 × 4.6 3 μm Supelcosil LC-8
Mobile phase: MeCN:50 mM phosphoric acid 45:55
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 5
Internal standard: mefenamic acid (8)
Limit of detection: 50-250 ng/mL

OTHER SUBSTANCES
Simultaneous: naproxen, flunixin, thiosalicylic acid, ethacrynic acid, phenylbutazone

KEY WORDS
plasma

REFERENCE
Singh,A.K.; Jang,Y.; Mishra,U.; Granley,K. Simultaneous analysis of flunixin, naproxen, ethacrynic acid, indomethacin, phenylbutazone, mefenamic acid and thiosalicyclic acid in plasma and urine by high-performance liquid chromatography and gas chromatography-mass spectrometry, *J.Chromatogr.*, **1991**, *568*, 351–361.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 100 μL Plasma + 400 μL 200 mM perchloric acid, centrifuge at 3000 g, inject a 20 μL aliquot of the supernatant. Urine. Dilute 1:1 with water, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 75 × 2.1 pellicular reverse phase (Chrompack no. 28653)
Column: 250 × 4.6 5 μm Cp Spherisorb ODS (Chrompack)
Mobile phase: Gradient; MeCN:5 g/L orthophosphoric acid from 80:20 to 60:40 (sic, probably 20:80 to 40:60) over 30 min then stay there for 5 min, then to initial conditions over 5 min, equilibrate for 2 min before next injection
Flow rate: 1.2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 28.13
Limit of detection: 5.3 ng/mL
Limit of quantitation: 146 ng/mL (urine), 60 ng/mL (plasma)

OTHER SUBSTANCES

Simultaneous: metabolites, glucuronides

KEY WORDS

plasma; pharmacokinetics; also details of preparative procedure

REFERENCE

Vree,T.B.; Van den Biggelaar-Martea,M.; Verwey-van Wissen,C.P.W.G.M. Determination of indomethacin, its metabolites and their glucuronides in human plasma and urine by means of direct gradient high-performance liquid chromatographic analysis. Preliminary pharmacokinetics and effect of probenecid, *J.Chromatogr.*, **1993**, *616*, 271–282.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 201.7

CHROMATOGRAM

Retention time: 21.748

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: 500 μL Plasma + 100 μL 5 mg/mL indomethacin in MeOH, filter (Sartorius SM 13243 ultrafiltration unit at 4000 g for 30 min). Add filtrate to a dry Chem Elut column (modified diatomaceous earth), leave 3 to 5 min, elute with 6 mL diethyl ether, evaporate eluant under a stream of nitrogen at room temperature, sonicate residue with 100 μL MeOH for 10 min, vortex, inject 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:100 mM pH 4.0 sodium acetate buffer 55:45
Flow rate: 1
Injection volume: 20
Detector: E, ESA Coulochem 5100A dual electrode with an ESA guard cell, + 0.65 V

CHROMATOGRAM
Retention time: 11.74
Internal standard: indomethacin

OTHER SUBSTANCES
Simultaneous: dipyridamole

KEY WORDS
plasma; indomethacin is IS

REFERENCE
Barberi-Heyob,M.; Merlin,J.L.; Pons,L.; Calco,M.; Weber,B. A sensitive isocratic liquid chromatography assay for the determination of dipyridamole in plasma with electrochemical detection, *J.Liq.Chromatogr.*, **1994**, *17*, 1837–1848.

SAMPLE
Matrix: formulations
Sample preparation: Reconstitute 1 mg indomethacin sodium trihydrate injection with 2 mL water, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.2 5 μm Ultrasphere ODS C18 (Beckman)
Mobile phase: MeCN:50 mM H_3PO_4 60:40
Flow rate: 1
Injection volume: 5
Detector: UV 260

CHROMATOGRAM
Retention time: 6.5-7.0

KEY WORDS
injections

REFERENCE
Walker,S.E.; Gray,S.; Schmidt,B. Stability of reconstituted indomethacin sodium trihydrate in original vials and polypropylene syringes, *Am.J.Health-Syst.Pharm.*, **1998**, *55*, 154–158.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 250 μL Microsomal incubation, 150 μL ice-cold MeCN and 2.5 ng ketoprofen, centrifuge. Extract the mixture with 4 mL ethyl acetate, centrifuge at 3000 rpm for 10 min, remove the organic fraction and evaporate it under a gentle stream of nitrogen at 40°. Dissolve the residue in 30 μL MeOH and dilute to 60 μL with water. Inject a 30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm RP-18 (Kanto Chemical, Tokyo)

Mobile phase: MeCN:water 40:60 containing 0.6% acetic acid
Column temperature: 35
Flow rate: 1
Injection volume: 30
Detector: UV 260

CHROMATOGRAM
Retention time: 49.0
Internal standard: ketoprofen (18.0)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
liver; pharmacokinetics

REFERENCE
Nakajima,M.; Inoue,T.; Shimada,N.; Tokudome,S.; Yamamoto,T.; Kuroiwa,Y. Cytochrome P450 2C9 catalyzes indomethacin O-demethylation in human liver microsomes, *Drug Metab.Dispos.*, **1998**, *26*, 261–266.

SAMPLE
Matrix: perfusate
Sample preparation: Mix 100 nL perfusate with 600 nL perfusion fluid containing IS, inject an aliquot. (Perfusion fluid contained 104 mM NaCl, 25 mM sodium bicarbonate, 2.3 mM sodium biphosphate, 10 mM sodium acetate, 1.2 mM calcium chloride, 1 mM magnesium sulfate, 5 mM KCl, 5 mM dextrose, and 5 mM alanine.)

HPLC VARIABLES
Column: 300 × 2 10 μm μBondapak C18
Mobile phase: MeOH:water 52:48
Flow rate: 0.13
Injection volume: 0.2
Detector: F ex 295 em 376 following post-column reaction. The column effluent mixed with 4 M NaOH pumped at 0.0013 mL/min and the mixture flowed through a 130 μL PTFE coil at 64° to the detector.

CHROMATOGRAM
Internal standard: phenylbutazone
Limit of detection: 25 ng/mL

KEY WORDS
post-column reaction; microbore

REFERENCE
De Zeeuw,D.; Leinfelder,J.L.; Brater,D.C. Highly sensitive measurement of indomethacin using a high performance liquid chromatographic technique combined with post column in-line hydrolysis, *J.Chromatogr.*, **1986**, *380*, 157–162.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 40:60 adjusted to pH 5.5 with NaOH
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 264

OTHER SUBSTANCES
Also analyzed: carbamazepine, fenbufen, ketoprofen, α.-naphthoquinone, naproxen, tolmetin

REFERENCE

Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 OmniPac PAX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:10 mM sodium carbonate 18:82. B was MeCN:50 mM sodium carbonate 33:67. A:B from 100:0 to 0:100 over 10 min.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: 18

OTHER SUBSTANCES

Simultaneous: tolmetin, aspirin, ibuprofen, fenbufen, naproxen, carprofen, diflunisal

REFERENCE

Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE

Matrix: solutions
Sample preparation: Sample + 400 μL 5 mM DBD-PZ + 70 mM diethylphosphorocyanidate in MeCN, react for 6 h, inject a 1 μL aliquot. (Synthesis of 4-(N,N-dimethylaminosulfonyl)-7-N-piperazino-2,1,3-benzoxadiazole (DBD-PZ) is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei (TCI America, Portland OR). Add 123 mg 4-(N,N-dimethyl-

aminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to 129 mg piperazine in 20 mL MeCN at room temperature, stir for 30 min, evaporate under reduced pressure, dissolve residue in 50 mL 5% HCl, wash three times with 20 mL ethyl acetate, discard ethyl acetate extracts, adjust pH of aqueous solution to 13-14 with 5% NaOH, extract five times with 50 mL ethyl acetate, combine extracts, wash with 20 mL water, dry over anhydrous sodium sulfate, evaporate under vacuum to give 4-(N,N-dimethylaminosulfonyl)-7-N-piperazino-2,1,3-benzoxadiazole as orange crystals (mp 121-2°).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:water 65:35
Column temperature: 40
Flow rate: 1
Injection volume: 1
Detector: F ex 437 em 561

CHROMATOGRAM

Retention time: 8
Limit of detection: 3.9 fmol

OTHER SUBSTANCES

Simultaneous: ibuprofen

REFERENCE

Toyo'oka,T.; Ishibashi,M.; Takeda,Y.; Nakashima,K.; Akiyama,S.; Uzu,S.; Imai,K. Precolumn fluorescence tagging reagent for carboxylic acids in high-performance liquid chromatography: 4-substituted-7-aminoalkylamino-2,1,3-benzoxadiazoles, *J.Chromatogr.*, **1991**, *588*, 61–71.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol,

mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 125 × 3 Ecocart LiChrospher 100 RP-18
Mobile phase: Isopropanol:100 mM KH_2PO_4:formic acid 54:100:0.1
Flow rate: 0.6
Detector: UV 254

CHROMATOGRAM

Retention time: 10.7
Limit of quantitation: 200-500 ng/mL

KEY WORDS

solutions acemetacin; diclofenac; flurbiprofen; lonazolac; ketoprofen; naproxen; piroxicam; sulindac; tenoxicam

REFERENCE

Baeyens,W.R.G.; Van Der Weken,G.; Van Overbeke,A.; Zhang,Z.D. Preliminary results on the LC-separation of non-steroidal anti-inflammatory agents in conventional and narrow-bore RP set-ups applying columns with different internal diameters, *Biomed.Chromatogr.*, **1995**, *9*, 261–262.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeCN:water 45:55, pH adjusted to 3.5 with acetic acid
Detector: UV 280

CHROMATOGRAM

Internal standard: clomethacin

OTHER SUBSTANCES

Also analyzed: diclofenac, phenylbutazone

REFERENCE
Guterres,S.S.; Fessi,H.; Barratt,G.; Puisieux,F.; Devissaguet,J.-P. Poly(D,L-lactide) nanocapsules containing non-steroidal anti-inflammatory drugs: Gastrointestinal tolerance following intravenous and oral administration, *Pharm.Res.*, **1995**, *12*, 1545–1547.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 8.45 (A), 9.22 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 250 ng/mL solution in pH 2.5 buffer.

HPLC VARIABLES
Column: 150 × 0.32 3 μm Hypersil C18
Mobile phase: MeCN:pH 6.0 acetate/citrate buffer 45:55
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 14
Limit of detection: 0.4 ng/mL
Limit of quantitation: 1.2 ng/mL

KEY WORDS
microcolumn

REFERENCE
Streel,B.; Ceccato,A.; Chiap,P.; Hubert,P.; Crommen,J. Injection-generated solvent and pH gradients for sample enrichment on injection of large volumes in microcolumn liquid chromatography, *Biomed.Chromatogr.*, **1995**, *9*, 254–256.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 10 μm Spherisorb silica
Column: 120 × 2 5 μm Spherisorb ODS
Mobile phase: MeOH:20 mM pH 7.0 phosphate buffer 58:42
Column temperature: 30
Flow rate: 0.378
Injection volume: 10
Detector: UV 254

REFERENCE
Lough,W.J.; Mills,M.J.; Maltas,J. Analyte adsorption in liquid chromatography valve injectors for samples in non-eluting solvents, *J.Chromatogr.A*, **1996**, *726*, 67–75.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.45 μm), dilute the filtrate with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:10 mM pH 4 acetate buffer 50:50
Detector: UV 242

REFERENCE
Okimoto,K.; Rajewski,R.A.; Uekama,K.; Jona,J.A.; Stella,V.J. The interaction of charged and uncharged drugs with neutral (HP-β-CD) and anionically charged (SBE7-β-CD) β-cyclodextrins, *Pharm.Res.*, **1996**, *13*, 256–264.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2

Injection volume: 20
Detector: UV 254

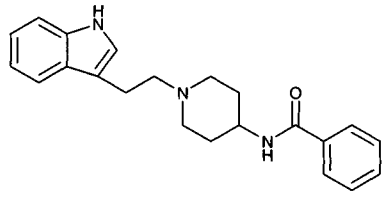
CHROMATOGRAM
Retention time: k' 0.58

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Indoramin



Molecular formula: $C_{22}H_{25}N_3O$
Molecular weight: 347.46
CAS Registry No.: 26844-12-2
Merck Index: 5000
Lednicer No.: 2 344

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

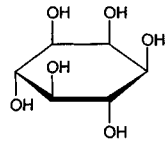
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 12.533

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Inositol



Molecular formula: $C_6H_{12}O_6$
Molecular weight: 180.16
CAS Registry No.: 87-89-8, 573-35-3 (monophosphate)
Merck Index: 5008

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 8.7 Aminex HPX-87C Ca^{++} (Bio-rad)
Mobile phase: Water
Column temperature: 50
Flow rate: 0.6
Detector: E, pulsed amperometric detector (Dionex ?), E1 0.1 V, T1 300 ms, E2 0.6 V, T2 120 ms, E3 -0.8 V, T3 300 ms, following post-column reaction. The column effluent mixed with 100 mM NaOH pumped at 0.2 mL/min and the mixture flowed to the detector.

CHROMATOGRAM
Retention time: 13.64 (myo-inositol), 14.16 (chiro-inositol), 11.16 (scyllo-inositol), 19.58 (neo-inositol)

OTHER SUBSTANCES
Also analyzed: 2-deoxygalactitol, 2-deoxyribositol, dextrose, fucitol, fucose, galactitol, galactose, mannitol, mannose, perseitol, sorbitol

KEY WORDS
post-column reaction

REFERENCE
Wang,W.T.; Safar,J.; Zopf,D. Analysis of inositol by high-performance liquid chromatography, *Anal.Biochem.*, **1990**, *188*, 432–435.

Insulin

Molecular formula: $C_{257}H_{383}N_{65}O_{77}S_6$(human)
Molecular weight: 5807.69 (human)
CAS Registry No.: 9004-10-8 (injection), 8049-62-5 (zinc suspension), 11061-68-0 (human), 12584-58-6 (pig), 11070-73-8 (cow), 9004-14-2 (neutral insulin), 8049-62-5 (isophane insulin), 9004-17-5 (protamine zinc suspension)
Merck Index: 5011

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 250 × 4 5 μm Nucleosil RP18
Mobile phase: MeCN:buffer 24:76 (w/w) (Prepare buffer by dissolving 9.8 g 85% phosphoric acid and 28.4 g sodium sulfate in 1 L water.)
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 214

CHROMATOGRAM
Retention time: 10.89

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Kunkel,A.; Günter,S.; Dette,C.; Wätzig,H. Quantitation of insulin by capillary electrophoresis and high-performance liquid chromatography. Method comparison and validation, *J.Chromatogr.A*, **1997**, *781*, 445–455.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 Synchropak C4
Mobile phase: Gradient. A was 0.05% trifluoroacetic acid in water. B was 0.05% trifluoroacetic acid in MeCN. A:B from 74:26 to 38:62 over 15 min.
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 4.7

REFERENCE
Ho,H.-O.; Hsiao,C.-C.; Sheu,M.-T. Preparation of microemulsions using polyglycerol fatty acid esters as surfactant for the delivery of protein drugs, *J.Pharm.Sci.*, **1996**, *85*, 138–143.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax 300 Å SB-C3
Mobile phase: Gradient. A was MeCN:water:trifluoroacetic acid 5:95:0.1. B was MeCN:water:trifluoroacetic acid 5:95:0.085. A:B from 85:15 to 47:53 over 20 min.
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: UV 215

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: angiotensin II, carbonic anhydrase, cytochrome C, leucine enkephalin, lysozyme, myoglobin, RNAase

REFERENCE
Ricker,R.D.; Sandoval,L.A.; Permar,B.J.; Boyes,B.E. Improved reversed-phase high performance liquid chromatography columns for biopharmaceutical analysis, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 93–105.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Protein & Peptide C18 (Vydac)
Mobile phase: MeCN:buffer 26:74 (Buffer was 28.4 g sodium sulfate and 2.7 mL phosphoric acid in 1 L water, pH adjusted to 2.3 with ethanolamine (if necessary).)
Column temperature: 40
Flow rate: 0.8
Detector: UV 214

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Yomota,C.; Yoshii,Y.; Takahata,T.; Okada,S. Separation of B-3 monodesamidoinsulin from human insulin by high-performance liquid chromatography under alkaline conditions, *J.Chromatogr.A*, **1996**, *721*, 89–96.

Interferon

Molecular formula: $C_{860}H_{1353}N_{227}O_{255}S_9$
Molecular weight: 19241.28
CAS Registry No.: 76543-88-9 (αA), 99210-65-8 (α2B), 98059-61-1 (gamma-1B)
Merck Index: 5015
Lednicer No.: 4 1

SAMPLE
Matrix: solutions
Sample preparation: Terminate the enzymatic hydrolysis by adding 10 μL 1% trifluoroacetic acid, 100 μL 8 M guanidine hydrochloride, inject an aliquot of the enzymatic digests.

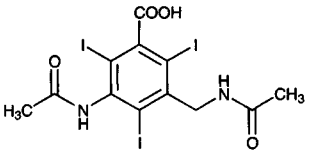
HPLC VARIABLES
Column: 150 × 4.6 5 μm Vydac C18
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid. B was MeCN:water 90:10 containing 0.1% trifluoroacetic acid. A:B from 52:48 to 45:55 in 45 min, to 20:80 over 2.5 min.
Flow rate: 0.6
Detector: UV 214

CHROMATOGRAM
Retention time: 20

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Gitlin,G.; Tsarbopoulos,A.; Patel,S.T.; Sydor,W.; Pramanik,B.N.; Jacobs,S.; Westreich,L.; Mittelman,S.; Bausch,J.N. Isolation and characterization of a monomethioninesulfoxide variant of interferon α-2b, *Pharm.Res.*, **1996**, *13*, 762–769.

Iodamide



Molecular formula: $C_{12}H_{11}I_3N_2O_4$
Molecular weight: 627.94
CAS Registry No.: 440-58-4, 18656-21-8 (meglumine salt)
Merck Index: 5031

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 100 µL 500 µg/mL iodopyracet in water + 5 mL MeOH, centrifuge at 1500 g for 20 min. Remove the supernatant and evaporate it to dryness under a stream of air at 65°, reconstitute the residue in 300 µL MeOH:water 90:10, let stand for 10 min, inject a 10 µL aliquot. Urine. 100 µL Urine + 900 µL mobile phase + 100 µL 500 µg/mL iodopyracet in water, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 5 µm LiChrosorb RP-8
Mobile phase: MeOH:water 85:15 containing 10 mM tetrabutylammonium hydrogen sulfate and 10 mM Tris
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 8
Internal standard: iodopyracet (11)
Limit of detection: 200 ng/mL

KEY WORDS

plasma

REFERENCE

Hekman,P.; Van Ginneken,C.A. Rapid determination of renal contrast media in biological fluids by means of high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *182*, 492–495.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 0.5-1 mL Plasma + 1 mL 1 M HCl + 5 mL ethyl acetate, shake for 10 min, centrifuge, repeat extraction. Combine the organic layers and evaporate them under a stream of nitrogen at 60°, add 3 mL 100 mM NaOH, shake for 10 min, centrifuge, discard the organic layer. Remove the aqueous layer and add it to 500 µL 1 M HCl, add 5 mL ethyl acetate, shake for 10 min, centrifuge, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 µL mobile phase, inject a 10-20 µL aliquot. Urine. 100 µL Urine + 1 mL 1 M HCl + add 5 mL ethyl acetate, shake for 10 min, centrifuge, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 400 µL mobile phase, inject a 10-20 µL aliquot.

HPLC VARIABLES

Column: 250 × 2.6 10 µm ODS-HC Sil-X-1 (Perkin-Elmer)
Mobile phase: MeCN:water:85% phosphoric acid 4:96:0.03
Flow rate: 1
Injection volume: 10-20
Detector: UV 235

CHROMATOGRAM

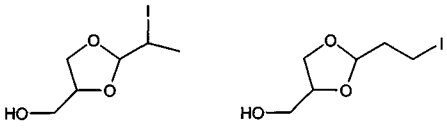
Retention time: 2.5
Internal standard: iodamide

OTHER SUBSTANCES
Extracted: o-iodohippurate, iothalamate

KEY WORDS
plasma; iodamide is IS

REFERENCE
Boschi,S.; Marchesini,B. High-performance liquid chromatographic method for the simultaneous determination of iothalamate and o-iodohippurate, *J.Chromatogr.*, **1981**, *224*, 139–143.

Iodinated glycerol



Molecular formula: $C_6H_{11}IO_3$
Molecular weight: 258.06
CAS Registry No.: 5634-39-9
Merck Index: 5033

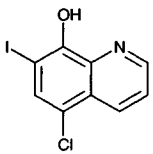
SAMPLE
Matrix: bulk
Sample preparation: Prepare an aqueous solution, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm Guard-pak (Waters)
Column: 250 × 4.6 5 μm Ultrasphere ODS C18
Mobile phase: MeCN:water 5:95
Flow rate: 1
Detector: RI

KEY WORDS
this procedure determines glycerol, a component of iodinated glycerol

REFERENCE
Cannon,J.M.; Brown,R.D.; Murrill,E.M.; Jameson,C.W. Identification of components in iodinated glycerol, *J.Pharm.Sci.*, **1989**, *78*, 48–51.

Iodochlorhydroxyquin



Molecular formula: C_9H_5ClINO
Molecular weight: 305.50
Merck Index: 5052

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL concentrated perchloric acid, vortex for 30 s, centrifuge at 15° at 5000 rpm for 15 min. Remove 500 μL of the supernatant and add it to 5 mL ether, vortex for 10 s, centrifuge at 15° for 10 min, repeat extraction. Add 10 mL ether to the original precipitate, vortex for 1 min, centrifuge at 15° at 5000 rpm for 15 min. Combine the extracts and dry them over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen at 38°, reconstitute the residue in 500 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 40 × 5 RP-18-MPLC (Brownlee)
Column: 250 × 2.6 ODS-HC-SIL-X-I (Perkin-Elmer)

Mobile phase: MeOH:50 mM phosphoric acid 80:20 (Flush column with MeOH at the end of each day.)
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 256

CHROMATOGRAM
Retention time: 5
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES
Noninterfering: hydrocortisone

KEY WORDS
plasma

REFERENCE
Ezzedeen,F.W.; Masoud,A.N.; Stohs,S.J.; Lerman,S.J. High-performance liquid chromatographic analysis of iodochlorhydroxyquin in plasma, *J.Pharm.Sci.*, **1981**, *70*, 889–891.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Weigh out 30 mg of bulk drug or an amount of cream equivalent to 30 mg iodochlorhydroxyquin, add 70 mL THF, shake vigorously until the cream has dissolved, make up to 100 mL with THF. Remove a 5 mL aliquot and add it to 1 mL pyridine and 1 mL acetic anhydride, heat at 60° for 15 min, cool, add 15 mL 450 μg/mL testosterone acetate in 94:6 butyl chloride:THF, mix thoroughly. Remove a 3 mL aliquot and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 15 mL mobile phase with gentle warming and vigorous shaking, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μPorasil
Mobile phase: Butyl chloride:water-saturated butyl chloride:THF:glacial acetic acid 55:55:3:2
Flow rate: 2-3
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: testosterone acetate (8)

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
cream; normal phase; derivatization; acetylation

REFERENCE
Kubiak,E.J.; Munson,J.W. Analysis of iodochlorhydroxyquin in cream formulations and bulk drugs by high-performance liquid chromatography, *J.Pharm.Sci.*, **1982**, *71*, 872–875.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Weigh out ointment, cream, or bulk drug containing 30 mg of iodochlorhydroxyquin, add 40-50 mL THF, dissolve with heating, make up to 100 mL with THF. Remove a 5 mL aliquot and add it to 1 mL 10 mg/mL nickel chloride in MeOH, add 5 mL 0.4 mg/mL diphenylamine in MeOH, make up to 50 mL with MeOH, filter, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak phenyl

Mobile phase: MeCN:MeOH:water 30:20:50 containing 1 mM $NiCl_2$
Flow rate: 1.2
Detector: UV 273

CHROMATOGRAM
Retention time: 7.5
Internal standard: diphenylamine (11)

OTHER SUBSTANCES
Simultaneous: chloroxine, iodoquinol

KEY WORDS
ointment; creams; separated as Ni chelates

REFERENCE
Wojtowicz,E.J. Reverse-phase high-performance liquid chromatographic determination of halogenated 8-hydroxyquinoline compounds in pharmaceuticals and bulk drugs, *J.Pharm.Sci.*, **1984**, *73*, 1430–1433.

SAMPLE
Matrix: formulations
Sample preparation: Ointment. 50 mg Ointment + 10 mL ether, vortex until dissolved. Remove a 200 μL aliquot and add phenyl salicylate in mobile phase, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 10 mL mobile phase, warm for 1 min on a steam bath, vortex for 1 min, cool. Remove an aliquot, dilute with mobile phase, inject an aliquot. Cream. Suspend 50 mg cream in 10 mL mobile phase by vortexing. Remove an aliquot and add phenyl salicylate in mobile phase, evaporate to dryness under a stream of nitrogen at 40°, suspend the residue in 10 mL mobile phase, warm for 1 min on a steam bath, vortex for 1 min, cool. Remove an aliquot, dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 40 × 5 RP-18-MPLC (Brownlee)
Column: 250 × 2.6 ODS-HC-SIL-X (Perkin-Elmer)
Mobile phase: MeOH:50 mM phosphoric acid 70:30 (Flush column with MeOH at the end of each day.)
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 256

CHROMATOGRAM
Retention time: 7.5
Internal standard: phenyl salicylate (5.25)

OTHER SUBSTANCES
Simultaneous: hydrocortisone

KEY WORDS
ointment; cream

REFERENCE
Ezzedeen,F.W.; Stohs,S.J.; Masoud,A.N. High-performance liquid chromatographic analysis of iodochlorhydroxyquin and hydrocortisone in ointments and creams, *J.Pharm.Sci.*, **1983**, *72*, 1036–1039.

SAMPLE
Matrix: feces, tissue, urine
Sample preparation: Urine. 1-5 mL Urine + 1-5 mL diethyl ether, vortex for 10 s, centrifuge at 15° at 3000 g for 10 min. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in mobile phase, inject a 20 μL aliquot. (Hydrolyze conjugates by adjusting pH to 5 with 1 M acetate buffer. add β-glucuronidase (Sigma) to a final concentration of 200 U/mL, heat at 37° for 2 h, neutralize with 3 M NaOH, add 1-5 mL diethyl ether, vortex for 10 s, centrifuge at 15° at 3000

g for 10 min. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen at 40°, reconstitute in 500 μL benzene (Caution! Benzene is a carcinogen!), add to an alumina column, wash with 2 mL benzene:pyridine 7:1, wash with 2 mL acetone, wash with 2 mL 100 mM acetic acid in MeOH, wash with 2 mL MeOH. Remove the alumina and add it to 1 mL saturated aqueous sodium fluoride, extract twice with 5 mL diethyl ether. Combine the extracts and evaporate them to dryness, reconstitute the residue in mobile phase, inject a 20 μL aliquot.) Tissue. Homogenize (Potter-Elvehjem) 1 g liver and 2 mL mobile phase, place homogenate in another tube, rinse original tube with 1 mL mobile phase, add rinse to homogenate, add 5 mL diethyl ether, vortex for 1 min, centrifuge at 3000 g for 10 min, repeat extraction twice. Combine the organic layers and evaporate them to dryness, reconstitute in 500 μL benzene (Caution! Benzene is a carcinogen!), add to an alumina column, wash with 2 mL benzene:pyridine 7:1, wash with 2 mL acetone, wash with 2 mL 100 mM acetic acid in MeOH, wash with 2 mL MeOH. Remove the alumina and add it to 1 mL saturated aqueous sodium fluoride, extract twice with 5 mL diethyl ether. Combine the extracts and evaporate them to dryness, reconstitute the residue in mobile phase, inject a 20 μL aliquot. Feces. Homogenize (Potter-Elvehjem) 1 g feces and 5 mL mobile phase, adjust pH to 5 with 1 M acetate buffer, add β-glucuronidase (Sigma) to a final concentration of 200 U/mL, heat at 37° for 2 h, neutralize with 3 M NaOH, add 1-5 mL diethyl ether, vortex for 10 s, centrifuge at 15° at 3000 g for 10 min. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen at 40°, reconstitute in 500 μL benzene (Caution! Benzene is a carcinogen!), add to an alumina column, wash with 2 mL benzene:pyridine 7:1, wash with 2 mL acetone, wash with 2 mL 100 mM acetic acid in MeOH, wash with 2 mL MeOH. Remove the alumina and add it to 1 mL saturated aqueous sodium fluoride, extract twice with 5 mL diethyl ether. Combine the extracts and evaporate them to dryness, reconstitute the residue in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 40 × 5 RP-18-MPLC (Brownlee)
Column: 250 × 2.6 ODS-HC-SIL-X-I (Perkin-Elmer)
Mobile phase: MeOH:50 mM phosphoric acid 70:30 (Flush column with MeOH at the end of each day.)
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 256

CHROMATOGRAM

Retention time: 7.5
Limit of detection: 200 ng
Limit of quantitation: 250 ng/g (feces), 500 ng/g (liver), 200 ng/mL (urine)

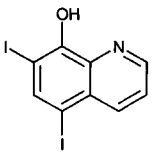
KEY WORDS

liver; dog; SPE

REFERENCE

Ezzedeen,F.W.; Stohs,S.J.; Stublar,M. Analysis of iodochlorhydroxyquin in biological materials by high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *276*, 121–128.

Iodoquinol



Molecular formula: $C_9H_5I_2NO$
Molecular weight: 396.95
CAS Registry No.: 83-73-8
Merck Index: 5063

SAMPLE

Matrix: bulk, formulations
Sample preparation: Cream, bulk. Weigh out cream or bulk drug containing 20 mg of iodoquinol, add 40-50 mL THF, dissolve with heating, make up to 100 mL with THF. Remove a 5

mL aliquot and add it to 1 mL 10 mg/mL nickel chloride in MeOH, add 3 mL 0.4 mg/mL diphenylamine in MeOH, make up to 50 mL with MeOH, filter, inject an aliquot. Tablets. Finely powder tablets, weigh out amount equivalent to 200 mg iodoquinol, add 150 mL THF, haet on a steam bath for a few min, shake mechanically for 30 min, make up to 250 mL with THF. Remove a 25 mL aliquot and make up to 100 mL with THF. Remove a 5 mL aliquot and add it to 1 mL 10 mg/mL nickel chloride in MeOH, add 3 mL 0.4 mg/mL diphenylamine in MeOH, make up to 50 mL with MeOH, filter, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak phenyl
Mobile phase: MeCN:MeOH:water 30:20:50 containing 1 mM $NiCl_2$
Flow rate: 1.2
Detector: UV 273

CHROMATOGRAM
Retention time: 9
Internal standard: diphenylamine (11)

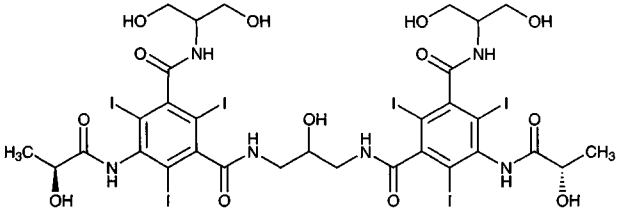
OTHER SUBSTANCES
Simultaneous: chloroxine, iodochlorhydroxyquin

KEY WORDS
tablets; creams; separated as Ni chelates

REFERENCE
Wojtowicz,E.J. Reverse-phase high-performance liquid chromatographic determination of halogenated 8-hydroxyquinoline compounds in pharmaceuticals and bulk drugs, *J.Pharm.Sci.*, **1984**, *73*, 1430–1433.

Iofratol

Molecular formula: $C_{31}H_{36}I_6N_6O_{13}$
Molecular weight: 1462.09
CAS Registry No.: 141660-63-1



SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Add 30 μL 2 mg/mL IS in water to 100 μL plasma. Add 30 μL 35% perchloric acid. Agitate and centrifuge at 3500 g for 10 min. Inject a 10 μL aliquot of the clear supernatant. Urine. Dilute 1 mL urine with 2 mL water, centrifuge at 4500 g for 15 min. Add 100 μL 5 mg/mL IS, 100 μL glacial acetic acid, and an ion-exchange resin mixture (1 g Duolite A-30B + 900 mg Amberlite IR-120). Dilute the suspension to 5 mL with water. Agitate for 30 min and centrifuge at 3500 g for 5 min. Inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 30 × 4 7 μm LiChrosorb RP-8
Column: 250 × 4.6 5 μm LiChrosorb RP-8
Mobile phase: MeCN:5 mM pH 4.5 potassium dihydrogen phosphate 5:95
Column temperature: 45
Flow rate: 1
Injection volume: 10
Detector: UV 242

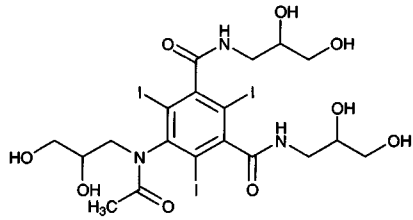
CHROMATOGRAM
Retention time: 12.0
Internal standard: iopamidol (4.1)
Limit of detection: 75 ng/mL (plasma), 430 ng/mL (urine)

KEY WORDS
plasma; rat; human

REFERENCE
Arbughi,T.; Bertani,F.; Celeste,R.; Grotti,A.; Tirone,P. High-performance liquid chromatographic determination of the X-ray imaging contrast agent, iofratol, in plasma and urine, *J.Chromatogr.B*, **1997**, *701*, 103–113.

Iohexol

Molecular formula: $C_{19}H_{26}I_3N_3O_9$
Molecular weight: 821.14
CAS Registry No.: 66108-95-0
Merck Index: 5068



SAMPLE
Matrix: blood
Sample preparation: Mix serum with an equal volume of MeCN, vortex for 15 s, centrifuge at 14000 g for 30 s, dilute the supernatant 100-fold with mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 40 × 3.2 3 μm Velosep RP-18 (Applied Biosystems)
Mobile phase: 8 mM pH 2 Phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 254

KEY WORDS
serum

REFERENCE
Shihabi,Z.K.; Constantinescu,M.S. Iohexol in serum determined by capillary electrophoresis, *Clin.Chem.*, **1992**, *38*, 2117–2120.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 50 μL 250 μg/mL acetaminophen in 100 mM HCl, add to SPE cartridge containing 150 mg 80-100 mesh Chromosorb P/NAW, elute with 1 mL ethyl acetate:MeOH 5:1, add the eluate to 50 μL 100 mM HCl, vortex for 15 s, centrifuge at 10000 g for 3 min, inject a 20 μL aliquot of the lower aqueous phase.

HPLC VARIABLES
Column: 5 μm C8
Mobile phase: MeCN:20 mM pH 3.3 phosphoric acid 2.5:97.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Internal standard: acetaminophen
Limit of detection: <1 μg/mL

OTHER SUBSTANCES
Extracted: aminohippuric acid (PAH)

KEY WORDS
serum; SPE